Volume : 2 | Issue : 11 | November 2013 • ISSN No 2277 - 8179

Research Paper

Prevalence of burn wound infections with aerobic and anaerobic bacteria and their antimicrobial susceptibility pattern at tertiary care hospital

Medical Science

KEYWORDS : Burn, Prevalence, Infections, antimicrobial susceptibility.

Ashrit.S.Bhat — Final year MBBS, Dept. of Microbiology, S. S. Institute of Medical Sciences and Research Centre, Davangere 577005, Karnataka. India

VinodKumar C.S. — Associate professor, Dept. of Microbiology, S. S. Institute of Medical Sciences and Research Centre, Davangere 577005, Karnataka. India

ABSTRACT

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care1. A prospective study was carried out in 30 burn patients admitted in Burn unit of S.S. Institute of Medical Sciences and Research Centre, Davangere, for over a period of four months to evaluate time-related changes in bacterial colonization and their sensitivity pattern.

Methods: Periodic swabs were taken from the burn wound on Day 0, Day 7, Day 14 and Day 21 to see the changing pattern of organisms during hospital stay of patients by standard microbiological techniques and antimicrobial susceptibility testing was done as per CLSI guidelines.

Results: In the present study burn injury was highest in the age group 20-30 yrs (42%). Female to male ratio was 1.4:1 Among the 120 samples, single organism was isolated in 64% samples and mixed organism in 26% and no growth in 12%. Among the single isolates A.baumannii was leading (28%) followed by Ps.aeruginosa (18%), K. pneumoniae (14%) and S.aureus (12%). Among mixed growth Ps.aeruginosa + A.baumanni (42%) and K.pneumoniae + Bacteriodes fragilis (36%) were leading combination of organism seen. There was time related changes in bacterial isolation from burn wound during hospital stay of patients. On admission 52% of the isolated organisms were S.aureus, only 18% of CoNS and 10% E. coli. These findings were gradually changing with time and on day 21 S.aureus were only 6% whereas A.baumannii was isolated in 28% and Ps.aeruginosa were isolated in 18%. Antimicrobial sensitivity test showed that Ps. aeruginosa and A.baumannii was highly resistant to antimicrobial agents.

Conclusion: It is crucial for every burn institution to determine the specific pattern of a burn wound microbial colonization, the time-related changes in dominant flora, and the antimicrobial sensitivity profiles so that hospital stay can be shortened thereby improving overall infection related morbidity and mortality.

Introduction

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality[1] It has been estimated that 75% of all deaths following thermal injuries are related to infection[2]. One of the key areas with which surgeons treating burn patients is concerned is septic complications, as burn wound is an ideal culture medium for microorganisms [3]. In addition, cross infection results between different burn patients due to overcrowding in burn wards. To establish any gains in infection control measures, it requires a brief understanding of wound bacteriology. It is very crucial for every burn institution to determine the specific pattern of burn wound microbial colonization, the time related changes in the dominant flora and the antimicrobial sensitivity profiles[4]. This becomes more important because of the fact that our hospital caters to majority of burn patients of this area. This study was carried out to document burn wound infection pattern in our setup so as to enable early treatment of imminent septic episodes with proper empirical systemic antibiotics.

Materials and methods

The study protocol was approved by Institutional ethical review board, S. S. Institute of Medical Sciences and Research Centre, NH-4, Davangere, Karnataka. Written or verbal consent of patient or legal guardian and permission of the respective authority of burn unit were taken.

Patients admitted within 24 hours of burn injury were included were as patients admitted after 24 hours of burn injury were excluded. The microbial colonization of wounds was studied weekly from the date of admission to the 21st day of hospitalization. This prospective study was done on 30 patients admitted in burn unit. A total of 120 surface swabs were taken using standard methods. On admission, the sampling procedure included collection of swab from clinically deep area of burn wound site prior to any cleansing[5,6]. Later swabs were taken on occasions of surgical debridement or surgical excision and grafting. In each sampling procedure, the bandages were removed, the remnants of topical antimicrobial agents were scraped away and the wounds were swabbed before washing and applying new topical antimicrobial agents[5]. Swabs were collected by using sterile cotton tipped swabs. Specimens were immediately transferred to sterile test tube. In case of collection of sample from dry surface, swabs were moistened with sterile normal saline. The specimens were transported in sterile, leak proof container to department of microbiology. Wound swabs obtained from the burn patients were subjected to microbiological analysis. All specimens were inoculated on 5% blood agar, Mac Conkey agar and chocolate agar plates and incubated overnight at 37^{0}C. The isolates were identified by standard microbiological techniques[6,7].

All the organisms isolated were subjected for antimicrobial susceptibility testing. It was carried out using Kirby Bauer's disc diffusion method for commonly used antibiotics[8].

Methicillin resistance was detected by disc diffusion technique using 30µg cefoxitin disc (Hi-Media, Mumbai)[9]. Tests for ESBL production was done by *Double disk approximation test* [10,11]. Amp C Disk Tests were also done to meropenem resistant strains for detection of AmpC β-lactamases [12]. Metallo β-lactamase (MBL) production was detected by Modified Hodge test[13,14]. EDTA disk synergy (EDS) test was done with simultaneous testing of two different ß-lactams (meropenem and ceftazidime) for detection of metallo-β-lactamases in the meropenem resistant isolates.

Result

Of the 30 burned patients, 20 (66.7%) were female and rest were male. The mean age was 28 ± 6 yr. In study group, 28 (93.3%) were directly admitted to our hospital and 02 (6.7%) were admitted from another hospital. Fire was the major cause of burn (58%) followed by electric burn (18%). The mean percentage of burn was 48.8% of total body surface area (TBSA). The mean length of hospital stay was 25 ± 17. The pattern of organisms cultured from the wounds show that a majority (70%) were Gram negative organisms, whereas Gram positive organisms contributed 30% in total. Among the 120 samples collected from 30 patients, single organism was isolated in 64% samples and mixed organism in 26%. Among single isolates *A.baumannii* was leading (28%) followed by *Ps.aeruginosa* (18%), *K. pneumoniae* (14%) and *Staphylococcus aureus* (12%) [Table-1]. Among mixed growth *Ps.aeruginosa* + *A.baumanni* (42%) and *K.pneumoniae* + *Bacteri-*

Volume : 2 | Issue : 11 | November 2013 • ISSN No 2277 - 8179

odes fragilis (36%) were leading combination of organism seen [Table 2]. There was time related changes in bacterial isolation from burn wound during hospital stay of patients.

Prospective study revealed time-related changes in the organism isolation. Gram positive organisms were initially prevalent then were gradually superceded by Gram negative organisms (Table- 3).

Mixed organisms were absent on admission culture which were gradually increasing up to Day 21. Isolation of *Staphylococcus aureus* was 20% on admission and was gradually decreased to 4.2% on Day 14th and absent on day 21st. On the other hand single isolation of *Pseudomonas aeruginosa* and *Proteus mirabilis* were 36% and 10% each on 7th day culture which were gradually decreased up to 35.7% for *Pseudomonas aeruginosa* and 6.3% for *Proteus mirabilis* respectively on day 21st and day 14th. While *Acinetobacter baumannii* was isolated in 4% on 7th day culture and increased to 16.7% on 14th day and 21.4% on 21st day.

The antibiogram of Gram negative organisms isolated from the burn wound is shown in Table-5. *Pseudomonas aeruginosa* was highly sensitive to Imipenem (66.7%) followed by Aztreonams (55.6%) and Piperacillin (55.6%) but resistance to Ceftriaxone and Ciprofloxacin was 100% followed by Cotrimoxazole (94.4%), Cefotaxime (94.4%), and Gentamycine (83.3%). Similarly *Klebsiella pneumoniae* was highly sensitive to imepenem (41.6%). Its resistance to Ampicillin was 91.6% followed by Ciprofloxacin (83.3%), Gentamicin (75.0%) and Cotrimoxazole (66.7%). *Proteus mirabilis, Enterobacter cloacae* and *Acinetobacter baumannii* were sensitive to Imipenem.

Antimicrobial sensitivity Pattern of Gram positive organisms isolated from burn wound is shown in Table-4. *Staphylococcus aureus,* CoNS and *Enterococci feacalis* were 100% sensitive to vancomycin Among *Staphylococcus aureus* 80% resistance to cephalexin, Gentamycin. 40% of the isolates were resistant to Cephoxitin (Methicillin resistant *Staphylococcus aureus*) Among CoNS, none of the isolates were resistant to Cephoxitin. Majority of CoNS were resistant to Gentamycin and Erythromycin. Among Enterococci feacalis, maximum resistance was seen for the drugs like Cephalexin (80%) and Ceftriaxone (80%).

Prevalence of beta lactamases among gram negative bacilli in burn patient is depicted in table 6. Prevalence of extended spectrum beta lactamase is 42.2%, Amp C mediated resistance is 10.4% and metallo beta lactamase mediated resistance is 15.6%

Distribution of beta lactamases among gram negative bacilli in burn patient is depicted in table 7. *Pseudomonas aeruginosa* is the predominant extended spectrum beta lactamase (22/48) and metallo beta lactamase (8/48) producer and *Acinetobacter baumannii* is the predominant Amp C producer (7/49)

Table 1: Incidence of mono aetiology among the burn cases

Organisms	Number	percentage
Acinetobacter baumannii	49	28.6
Pseudomonas aeruginosa	48	18.2
Klebsiella pneumonia	32	14.3
Staphylococcus aureus	20	13
Enterococcus feacalis	7	9.1
E.coli	6	7.7
Proteus mirabilis	4	5.2
CoNS	3	3.9

Table:2 Incidence of poly aetiology among the burn cases

Mixed growth	Number	Percentage
Ps.aeruginosa+ A.baumannii	24	41.4
K.pneumoniae+Bacteriodes fragilis	21	36.2
Ps.aeruginosa+ Staphylococcus aureus	10	17.2
A.baumannii+Bacteriodes fragilis	3	5.2

Table:3 Time-related changes in organism isolation from burn wound

Organisms	Total number of isolates	Days			
		0	7	14	21
Acinetobacter baumannii	22	No Growth	15	22	8
Pseudomonas aeruginosa	14	No Growth	14	12	5
Klebsiella pneumoniae	11	No Growth	8	11	6
Staphylococcus aureus	10	No Growth	9	10	Nil
Enterococcus feacalis	7	No Growth	5	7	1
E.coli	6	No Growth	4	6	Nil
Proteus mirabilis	4	No Growth	2	4	Nil
CoNS	3	No Growth	3	nil	Nil
Mixed growth	29	Nil	12	29	16
No growth	14	120 samples	48	19	84
Mortality	6	nil	nil	nil	6

Table: 4
Antibiotic sensitivity pattern of gram Positive negative bacteria isolated from burn patients

Antibiotics	Staphylococcus aureus		Enterococci faecalis		CoNS	
	No	%	No	%	No	%
Amikacin	16	80	5	71.4	1	33.3
Cefotaxime	18	90	6	85.7	1	33.3
Ceftriaxone	14	70	6	85.7	1	33.3
Tetracycline	16	80	5	71.4	1	33.3
Co-trimaxozole	15	75	7	100.0	1	33.3
Penicillin	20	100	7	100.0	2	66.7
Erythromycin	13	65	6	85.7	1	33.3
Gentamicin	15	75	6	85.7	1	33.3
Ampicillin +sulbactum	12	60	4	57.1	1	33.3
Cefoxitin	11	55		0.0		0.0
Netilmycin	14	70	6	85.7	1	33.3
Clindamycin	12	60	4	57.1	1	33.3
Linezolid	13	65	4	57.1	1	33.3

Table:5 Antibiotic sensitivity pattern of gram negative bacteria isolated from burn patients

Antibiotics	Acinetobacter		Pseudomonas		Klebsiella		E.coli	
	No	%	No	%	No	%	No	%
Amikacin	28	57.1	32	66.7	12	60.0	2	33.3
Cefotaxime	39	79.6	44	91.7	16	80.0	4	66.7
Ceftriaxone	42	85.7	45	93.8	18	90.0	4	66.7
Ciprofloxacin	37	75.5	46	95.8	17	85.0	4	66.7
Cotrimaxozole	33	67.3	40	83.3	14	70.0	4	66.7
Imipenem	18	36.7	22	45.8	9	45.0	2	33.3
Aztreonam	22	44.9	21	43.8	8	40.0	2	33.3

Piperacillin	34	69.4	39	81.3	14	70.0	4	66.7
Piperacillin +tazobactam	16	32.7	20	41.7	10	50.0	1	16.7
Ceftriaxone+ sulbactum	15	30.6	22	45.8	8	40.0	3	50.0
Sparfloxacin	29	59.2	44	91.7	18	90.0	4	66.7
Meropenam	16	32.7	18	37.5	9	45.0	2	33.3

Table6: Prevalence of Beta lactamases mediated resistance among burn patients

Beta lactamases	%
ESBL	42.2
AmpC	10.4
MBL	15.6

Table7 : Distribution of beta lactamases among different gram negative bacteria

Organisms	ESBL	AmpC	MBL
Pseudomonas aeruginosa	22	3	8
Acinetobacter baumannii	18	7	7
Klebsiella pneumoniae	14	3	4
E.coli	2	1	0
Proteus mirabilis	1	0	2

Discussion

Burn wound if not excised and grafted early becomes an ideal culture medium for the growth of microorganisms[8]. Infection is an important cause of morbidity and mortality in burns. Severe burn patients are very susceptible to infection because of wide exposed raw areas, the presence of necrotic tissue, protein rich exudates, inability of blood to reach the colonized areas of wounds and other host defense mechanisms[9]. The colonization and later invasion of tissues is from patient's normal flora of skin or from gastrointestinal tract or more usually by cross infection[10]. In the face of high mortality because of bacteraemia in burned patients, it is important to select antibiotics or combination of antibiotics with broad coverage for the usual pathogens. In a large number of patients this has to be empirical pending results of cultures[5]. As the type of bacteria and their sensitivity vary from place to place analysis of burn wound microbial colonization is to be performed so that the prophylactic and therapeutic regimens could be rationalized[6,8,11]. There are also time- related changes in burn wound microbial colonization. Different types of study on burn wound infection have been carried out in different countries of the world. Among them few were regarding time-related changes in bacterial colonization[12]. In India time related changes in burn wound infection were not included in the study because patients were unable to bear the cost of treatment.

Infection with one or more organisms was present in 98% of cases. The changes in bacterial spectrum are not unexpected because of cross infections, resistance to drugs and introduction of new bacteria from other places. Hence incidence and spectrum of infection varies from place to place and country to country due to different therapeutic and preventive policy. *Pseudomonas aeruginosa* isolation was maximum in our study in both single (36%) and mixed (9%) infection (Table I). These findings were consistent with those of other centers of different countries[6,8]. But in a previous study in our country isolation of *Staphylococcus aureus* was leading[10]. In the present study *Staphylococcus aureus* was the second most common organism isolated (17.5%) followed by *Klebsiella pneumoniae*

Analysing the results of four swabs taken from burn wound of each patient it was observe that by day 21 all the sample yielded growth. Gram negative organisms were predominant. All these changes were gradual from starting to the end of sample collection (Table II). On the day of admission (Day 0) no bacteria were isolated. On day 7th colonization by *Pseudomonas aeruginosa* was 36% followed by *Staphylococcus aureus* 20% and *Klebsiella pneumoniae* 10%. On Day 14, gram negative bacteria were the predominant. *Pseudomonas aeruginosa* was isolated in 31.3% followed by *Klebsiella pneumoniae* 25% and *Acinetobacter baumannii* 16.7%. On Day 21 *Pseudomonas aeruginosa* was the predominant followed by *Enterococci feacalis* (21.4%), *Citrobacter freundii* (14.3%) and *Klebsiella pneumoniae* (7.1%) On Day 0 there was no mixed growth which was 20% on Day 21. All the (100%) swabs yielded growth on Day 21. Present study revealed that Gram positive cocci *(Staphylococcus aureus)* were initially prevalent then were gradually superceded by Gram negative bacilli specially *Pseudomonas aeruginosa* throughout patients hospital stay of 21 days. The study results of various worker revealed that the bacteriology of burn infection has been changing from time to time and also the antimicrobial sensitivity pattern[13,14]. There are also time- related changes in burn wound microbial colonization. Gram positive cocci are initially prevalent then are gradually superceded by Gram negative bacilli throughout the patients hospital stay that have a greater propensity to invade[3,6,8].

Gram-negative organisms like *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* continues to be a menace to the ill, fragile and debilitated patients in the burn care units, and the serotypes isolated are often resistant to multiple antibiotics. Thus these organisms continue to be a nightmare to burn specialist, microbiologists and hospital administrators. Inadequate space, shortage of staff, high occupation rates, widespread use of antimicrobial agents and increased susceptibility of population, are responsible to early colonization and subsequent infection by virulent strains resulting in high morbidity and mortality.

These time-related changes have also been found in our study. Periodic reviews of patterns of isolation and susceptibility profiles of organisms infecting burn wounds are needed[15] in order to modify the preventive and therapeutic strategies. It is therefore essential for every burn institution to determine its specific pattern of burn wound microbial.

Conclusion

Burns are a very common injury. Serious burn injuries are excruciatingly painful and require special care to prevent infection and reduce the severity of scarring. They can cause lifelong disabilities and leave physical and emotional scar. It is quiet clear that infections are serious problem among burns patients. *Pseudomonas aeruginosa* has emerged as the commonest organism causing infection and is resistant to most of the antibiotics. To keep a check on burn wound infections it is important for every hospital to have a data on prevalent organisms and their antibiotic susceptibility pattern. This study should be done frequently to check the changing pattern of the organisms and their susceptibility pattern. Based on this, the hospital should formulate an effective antibiotic policy.

Acknowledgement:

Authors would like to acknowledge Indian Council of Medical Research for funding this project

REFERENCE

1. Santucci SG, Gobara S, Santos CR, Fontana C, Levin AS. Infections in a burn intensive care unit : experience of seven years. J Hosp Infect 2003; 53, 6-13. | 2. Bowser Wallace BH, Graves DB, Caldwell FT. An epidemiological profile and trend analysis of wound flora in burned children: 7 years experience. Burns Incl Therm inj 1984; 11 ,16-25. | 3. Ahmad M, Shahid Hussain, Ibrahim Khan, Malik SA. Pattern of Bacterial invasion in burn patients at the Pakistan institute of Medical sciences, Islamabad, Annals of burns and fire disasters.2006:Vol.19(1);23-28 | 4. Pruitt BA, Colonel MC, MC Manvs AD. Opportunistic infections in severely burnt patients. American JMed 1984;76, 146-54. | 5. Church, D., Elsayed, S., Reid, O., Winston, B., Lindsay, R. Burn Wound Infections. | Clinical Microbiology Reviews, 2006;19 (2):403 – 434. | 6. Kaushik R , Kumar S , Sharma R , Lal P .Bacteriology of burn wounds -the first three | years in a new burn unit at the Medical College , Chandigarh. Burns 2001; 27, 595-97. Agnihotri N , Gupta V, Joshi RM . Aerobic bacterial isolates from burn wound infections and their antibiograms-a five year study. Burns 2004;30,241-43. | 7. Singh NP, Goyal R, Manchanda V, et al. Changing trends in bacteriology of burns in | the burns unit, New Delhi. Burns 2003;29, 129-32.37 | 8. Bauer, Kirby, Sherri, Turkek. Antibiotic susceptibility testing methods. American | Journal of Clin Patho. 1966;45:493-498. | 9. Tayyaba B, Jamshed A, Soofia A, et al. Antibiotic sensitivity pattern in burn wound | cultures. J Surg Pakistan 2005;10, 8-11. | 10. French, G.L., Ling, J., Hui, Y.W. Determination of methicillin-resistance in Staphylococcus aureus by agar dilution and disc diffusion methods. Journal of Antimicrobial Chemotherapy 1987: 20; 599-608 | 11. Brun-Buisson C, Legrand P, Philippon A, Montravers F, Ansquer M, Duval J et al. Transferable enzymatic resistance to third-generation cephalosporins during a 39 nosocomial outbreak of multi resistant Klebsiella pneumoniae. Lancet 1987; 8(2):302-6. | 12. Coudron PE, Moland ES, Sanders CC. Occurrence and Detection of Extended-Spectrum ß-lactamases in members of the family Enterobacteriaceae at a Veterans Medical Center: Seek and You May Find. J Clin Microbiol 1997; 35(10):2593-7. | 13. Singhal S, Mathur T, Khan S, Upadhyay DJ. Evaluation of method for Amp C β-lactamases in gram negative clinical isolate from tertiary care hospital. Indian Journal of Medical Microbiology 2005;23(2):120-124 | 14. Black JA, Moland ES, Thomson KS. Amp C disk test for detection of plasmid mediated Amp C β-lactamases. Journal of Clin Microbiol 2005;43(7):3110-13. |